levels of miR-1 and miR-133 were significantly increased in HF myocytes compared to controls (2 and 1.6 fold accordingly). Western blotting showed that PP2A regulatory (b56alpha) and catalytic subunits, specific targets of miR-1 and miR-133 validated by luciferase-reporter assay, were decreased in HF cells. Analysis using phospho-specific antibodies confirmed that RyR2 phosphorylation at Ser-2814 was significantly increased in HF myocytes compared to controls. CaMKII inhibitory peptide reduced the frequency of spontaneous Ca waves in paced current-clamped HF myocytes to low control values. These finding suggest that altered levels of major muscle-specific microRNAs contribute to abnormal RyR2 function in HF by depressing localized phosphatase activity to the channel, thus leading to excessive phosphorylation of RyR2s.

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Voltage-Dependent Anion Channel 2 modulates Resting Calcium Sparks, but not Action Potential-Induced Global Calcium Signaling in Cardiac Myocytes

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Voltage-dependent anion channels (VDACs) are pore forming proteins predominantly found in the outer mitochondrial membrane and is thought to transport calcium ion (Ca^{2+}). In this study, we have investigated the possible role of type 2 VDAC (VDAC2) in cardiac Ca^{2+} signaling and Ca^{2+} sparks using a lentiviral knock-down (KD) technique and two-dimensional confocal Ca^{2+} imaging in immortalized autorhythmic adult atrial cells, HL-1. We confirmed high expression of VDAC2 protein in ventricular, atrial and HL-1 cells using Western blot analysis. Infection of HL-1 cells with VDAC2-targeting lentivirus reduced the level of VDAC2 protein to ~10%. Comparisons of autorhythmic Ca^{2+} transients between wild type (WT) and VDAC2 KD cells showed no significant change in the magnitude, decay, and beating rate of the Ca^{2+} transients. Caffeine (10 mM)-induced Ca^{2+} release, which indicates sarcoplasmic reticulum (SR) Ca^{2+} content, was not altered by VDAC2 KD. Interestingly, however, the intensity, width, and duration of the individual Ca^{2+} sparks were significantly increased by VDAC2 KD in resting conditions, with no change in the frequency of sparks. These results suggest that VDAC2 may suppress focal Ca^{2+} releases through ryanodine receptors in atrial myocytes under resting conditions. The results also indicate that VDAC2 may not regulate action potential-induced global Ca^{2+} signaling and SR Ca^{2+} loading.

3046-Pos Board B151

African Trypanosomes Increase Calcium Wave Frequency in Isolated Adult Rat Cardiomyocytes via Secretion of Cathepsin L

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African trypanosomes are blood-borne extracellular parasites which have recently been linked to cardiac dysfunction in ~70% of sleeping sickness patients. Although this may result from an indirect effect of the parasite (e.g. myocarditis), a direct effect of the parasite on the heart has not been investigated. Adult rat cardiomyocytes were incubated with trypanosome growth media containing *Trypanosoma brucei* Lister427 (30min). A population assay assessed the percentage of cells demonstrating Ca^{2+} waves within a 1min period. Incubation with live trypanosomes led to a significant increase in the percentage of cells demonstrating Ca^{2+} waves ($54.8 \pm 2.8\%$ *vs.* $79.2 \pm 5.1\%$; media *vs.* live trypanosomes, $P<0.05$; n=4294 and 3006 cells respectively). This effect was maintained when cells were incubated with supernatant (trypanosomes removed from media by centrifugation) ($77.3 \pm 2.9\%$; n=2131 cells). Separate experiments showed the supernatant effect was lost upon boiling ($83.7 \pm 1.8\%$ *vs.* $66.3 \pm 2.4\%$; supernatant *vs.* boiled supernatant, $P<0.05$; n=527 and 612 cells respectively). Results were confirmed in Fura2AM loaded, field stimulated (1Hz) rat cardiomyocytes perfused with media (37°C). Following 4 min supernatant perfusion, the frequency of Ca^{2+} waves in the inter-stimuli interval was significantly increased (0.02 ± 0.01 *vs.* 0.44 ± 0.07 waves/s; media *vs.* supernatant, $P<0.05$; n=10). Since the parasite induces a similar phenomenon in brain mono-epithelial cells via cathepsin-L cysteine protease, we examined the role of cathepsin-L in the above effect on cardiomyocytes. In separate experiments, supernatant+K11777 (specific inhibitor of cathepsin-L) completely abolished the ability of supernatant to increase Ca^{2+} wave probability (56.3 ± 5.1 *vs.* $49.1 \pm 5.7\%$; media *vs.* supernatant+K11777, $P>0.05$), whereas CA074 (specific inhibitor of cathepsin-B) had no effect on Ca^{2+} wave frequency. These data suggest trypanosomes interact with cardiomyocytes leading to increased Ca^{2+} wave production via cathepsin-L. This may contribute to the cardiac abnormalities observed in patients with trypanosomiasis.

3047-Pos Board B152

Calcium Handling in Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes

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Fibroblasts from human skin biopsies can be reprogrammed into pluripotent stem cells (iPSC), which can then be coaxed to differentiate into myocytes with cardiac-specific properties (iPSC-CMs). The field of iPSCs is still in its infancy, but it is increasingly clear that the excitation-contraction coupling (ECC) machinery of differentiating CMs undergoes proportionally incremental complexity and it remains to be seen whether it reaches complete maturity in cultured cells. We used patch-clamp and confocal Ca^{2+} imaging for a comparative assessment of ECC in human iPSC-CM and adult cardiomyocytes. In the latter, entry of Ca^{2+} through the L-type Ca^{2+} channel (I_{Ca}) triggers rapid, uniform release of Ca^{2+} from the sarcoplasmic reticulum (SR) via CICR. In iPSC-CMs at early stages of differentiation, the current-voltage relationship for I_{Ca} is remarkably similar to that of adult cardiomyocytes, indicating that the appearance of a "trigger" for contraction is an early event in the ontogenesis of ECC that doesn't hinder efficient generation of Ca^{2+} signals. However, primitive iPSC-CMs commonly exhibit a poorly developed SR, as assessed by their variegated response to caffeine and their great dependence on extracellular Ca^{2+} for contraction. Cells are mostly rounded and t-tubules are absent. As a result, $[Ca^{2+}]_i$ transient waveforms appear non-uniform and start at the periphery of the cell, as is expected of a Ca^{2+} front with focal initiation that propagates later to the interior of the cell. At more advanced stages of differentiation, iPSC-CMs display fairly uniform Ca^{2+} fronts, suggesting fast propagation of external Ca^{2+} signals to the interior of the cell. Thus, by this coarse functional estimate, it is expected that iPSC-CMs become accurate models of cardiomyopathies at late stages of differentiation, but the developmental characteristics of ECC is unclear and warrants a systematic approach, which we are currently performing.

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Impaired Calcium Signaling Refractoriness Contibutes to Increased Rate of Diastolic Calcium Waves in Myocytes from Post-Myocardial Infarction Hearts

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Spontaneous Ca^{2+} waves (SCWs) are recognized as important contributors to triggered arrhythmia. SCWs waves are thought to arise when $[Ca^{2+}]_{SR}$ reaches a certain threshold level, which might be reduced in cardiac disease as a consequence of sensitization of ryanodine receptors (RyR2s) to luminal Ca^{2+}. We investigated the mechanisms of SCW generation by simultaneous measurements of cytosolic and luminal Ca^{2+} in myocytes from normal and diseased hearts using a canine model of post-myocardial infarction (MI) tachyarrhythmia. The frequency of SCW, recorded during periodic pacing in the presence of β-adrenergic receptor agonist isoproterenol, was significantly higher in MI myocytes than in control. Rather than occurring at once upon reaching a final $[Ca^{2+}]_{SR}$, SCWs arose with a distinct time delay from the attainment of the maximum $[Ca^{2+}]_{SR}$ in both experimental groups. While the rate of $[Ca^{2+}]_{SR}$ recovery following the SR Ca^{2+} release was similar between the two myocyte types, the maximally attainable $[Ca^{2+}]_{SR}$ was lower, and the latency to SCW was shorter in MI myocytes compared to control. Both phosphorylation at the CAMKII site Ser-2814 and the level of oxidized thiols were higher in RyR2s from MI hearts than in control. The CAMKII inhibitor, KN93, or the reducing agent, mercaptopropionylglycine, reduced SCW frequency in MI myocytes. The MI-related alterations in myocyte Ca^{2+} cycling were mimicked by the RyR2 agonist, caffeine. These results indicate that attainment of a certain threshold $[Ca^{2+}]_{SR}$ is not a sufficient condition for the generation of SCWs and that Ca^{2+} signaling refractoriness that develops following release critically influences SCW occurrence in the diastolic period. We conclude that shortened Ca^{2+} signaling refractoriness due to RyR2s phosphorylation and oxidation is responsible for the increased rate of SCWs observed in MI myocytes.

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Inositol 1,4,5 Triphosphate (IP3) Receptors Activate Type 1 ryanodine Receptors to Mediate Ca^{2+} Sparks Signaling in Adult Mammalian Skeletal Muscle

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Ca^{2+} sparks are the elemental event of Ca^{2+} induced Ca^{2+} release (CICR) that originate from clustered ryanodine receptor Ca^{2+} release channels (RyR1) in mammalian striated muscles. Previously we found that application of transient osmotic stress to the intact skeletal muscle leads to a robust Ca^{2+} spark response that is restricted to the periphery of sarcolemmal membrane. Here we